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Phase I Trial of Extracellular Adenosine 5′-Triphosphate in Patients With Advanced Cancer

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Adenosine 5′-triphosphate (ATP) has antineoplastic activity in vitro and in murine tumor systems, but there are no data in humans defining its potential use as an antineoplastic agent. We conducted a Phase I study to determine the spectrum of toxicity, maximum safely tolerated dose (MTD), and pharmacokinetics of intravenous ATP. Fourteen men with advanced cancer received 96-hour infusions of ATP once monthly in doses ranging from 50 to 100 μg/kg/minute. Toxicity was assessed by standard National Cancer Institute (NCI) criteria, cardiac function was monitored serially by two-dimensional echocardiography, and whole blood ATP was measured serially in a subset of patients. ATP was generally well tolerated and no significant hematologic toxicity was noted. The dose-limiting toxicity was a cardiopulmonary reaction characterized by chest tightness and dyspnea that resolved within seconds of discontinuing ATP. Dose-limiting cardiopulmonary toxicity occurred in 3 of 3 patients at 100 μg/kg/minute, in 3 of 6 patients at 75 μg/kg/minute, and 4 of 11 patients at 50 μg/kg/minute. Whole blood ATP levels significantly increased with treatment, reaching a steady state by 24 hours and returning to or near baseline by 1 week after treatment. Plateau levels were 63%, 67%, and 116% above baseline at 50, 75, and 100 μg/kg/min, respectively. We conclude that prolonged infusions of ATP are feasible with acceptable toxicity and that 50 μg/kg/minute is both the MTD and the most appropriate dose rate for subsequent Phase II testing of 96-hour infusions of ATP in patients with advanced cancer. © 1996 Wiley-Liss, Inc.*

Key words: nonsmall cell lung cancer, pharmacokinetics, biomodulation, drug resistance

INTRODUCTION

Adenosine 5′-triphosphate (ATP) occupies a central role in physiologic chemistry as the predominant form of intracellular energy. The physiologic role of extracellular ATP is less clear but several lines of evidence suggest that increased levels of extracellular ATP may inhibit the growth of neoplastic cells and modulate the effects of chemotherapy.

Increased concentrations of extracellular ATP are cytotoxic by several mechanisms in cell culture. The exposure of tumor cells to extracellular ATP has been reported to cause the intracellular accumulation of ATP and the arrest of tumor cells in S-phase followed by cell death [1]. This has been related to altered membrane permeability of transformed cells in tissue culture. The effects of ATP on cell permeability and S-phase are limited to transformed cells, with the exception of cells possessing secretory functions [2]. Increased cell permeability appears to be due to the induction of pores in the tumor cell membrane after exposure to extracellular ATP. These pores are the result of damage to the gap junction protein connexin-43 [3], which is more exposed in neoplastic cells than in normal cells [4]. In breast cancer cell lines, extracellular ATP induced apoptosis, which was correlated with increased levels of intracellular calcium [5]. Induction of apoptosis by extracellular ATP has also been demonstrated in rat thymocytes [6]. Extracellular ATP has been shown to lyse human monocytic leukemia cells in tissue culture; this process is markedly augmented by interferon-gamma through upregulation of a specific ATP membrane receptor [7]. Extracellular ATP has been proposed as a possible mediator of cell-mediated cytotoxicity by cytotoxic T lymphocytes and natural killer cells [8], although resistance to ATP and cytotoxic T lymphocytes can be separated [9].

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Several murine tumor models are sensitive to ATP administered in vivo. For example, Rapaport and Fontaine [10] have demonstrated significant antitumor activity for both ADP and ATP in the CT26 colon adenocarcinoma in $CB6F_1$ mice. Daily intraperitoneal injections in large volumes of saline inhibited tumor growth and a few animals were apparently cured. Toxicity was minimal as assessed by body weight measurements and gross observation of the animals. In another study, the inhibition of Ehrlich tumor cells in vivo by extracellular ATP was related to selective intracellular glutathione depletion, suggesting an intracellular effect of extracellular ATP exposure [11].

There is preliminary evidence suggesting that extracellular ATP may be capable of reducing multidrug resistance to chemotherapy mediated by p-glycoprotein [12]. In ovarian cancer cell lines, extracellular ATP enhances intracellular penetration by doxorubicin resulting in chemosensitization [13].

These in vitro and in vivo studies suggest that the administration of ATP may serve as a new form of biomodulator in cancer treatment. Studies of ATP administered to animals and humans in the past for other reasons have provided a preliminary pharmacologic basis for studies in patients with cancer, but they are not sufficient to allow immediate testing of ATP for efficacy. We therefore initiated a Phase I study of ATP in patients with advanced cancer.

MATERIALS AND METHODS

ATP

Adenosine-5′triphosphate (ATP-disodium) was obtained from MEDCO Research, Inc. in 2.0 g vials. The drug was reconstituted with 250 ml of 0.5 normal saline in glass bottles and infused through a peripheral vein over 6 hours using an Ivac infusion device. A new vial was used approximately every 6 hours and the total infusion time was 96 hours per course of therapy.

Patients

Patients with biopsy or cytology-proven advanced carcinomas who were not candidates for standard therapy were considered eligible for this study if they had a Karnofsky performance status (KPS) $\geq$50, a life expectancy of $\geq$6 weeks, 3 weeks or longer since the last chemotherapy or radiation therapy with resolution of any myelosuppression, adequate hepatic, renal, and bone marrow function as defined by white blood cell count $\geq$4,000/μl, platelet count $\geq$100,000/μl, serum creatinine $\leq$1.5 mg/100 ml, blood urea nitrogen $\leq$25 mg/100 ml, serum glutamic oxaloacetic transaminase less than 3 times normal, and serum bilirubin $<$2.0 mg/100 ml. The left ventricular ejection fraction had to be more than 50% by two-dimensional echocardiography and the patient had to have adequate pulmonary function as defined by a forced expiratory volume in one second (FEV_1) $\geq$50 percent of predicted and arterial blood gas measurements that were within normal limits. Patients with second or third degree atrioventricular block or sinus node dysfunction, those with bronchospastic disease, and those known to be hypersensitive to adenosine were excluded. Patients with nonsmall cell lung cancer had to have American Joint Committee on Cancer/World Health Organization stages IIIB or IV to be eligible. The study was approved by the institutional review board (IRB) and patients were required to sign an approved consent form before study entry.

Fourteen patients participated in this Phase I study of ATP treatment (Table I). All patients were male; the mean age was 62 years. Eight of the patients had nonsmall cell lung cancer. A single case of each of the following carcinomas comprised the remainder of the study group: gastric, esophageal, colon, hepatocellular, maxillary sinus, and prostate. All eligible, consenting patients were treated, all are evaluable for toxicity, and none were lost to follow-up. Six patients discontinued the study prematurely because of progression of disease (patients 7, 12, and 14) and intolerable side effects (patients 3, 4, and 11). There were seven protocol violations, as follows: discontinuous infusion of ATP (two patients); failure to record the pretreatment KPS (one patient); preexisting chronic obstructive pulmonary disease being treated with a bronchodilator (one patient); pretreatment borderline leukopenia (one patient); and pretreatment left ventricular ejection fractions that were equal to or less than 50% (two patients). None of these violations were thought to compromise the interpretation of the study results.

Schedule of Drug Administration

We chose to study a 96-hour infusion of ATP, rather than a shorter infusion schedule, because of our anticipation that the maximal benefit of ATP would be from its use in combination with other drugs, rather than as a single agent. A moderately prolonged infusion of this type would allow the addition of ATP to any of a large number of regimens extending as long as 5 days.

The study called for treatment of the first three patients with ATP given as a 96-hour continuous infusion at a dose rate of 50 μg/kg/minute. If well tolerated, subsequent dose escalations were planned in groups of three patients in increments of 25 μg/kg/minute until the maximally tolerated dose (MTD) was reached, as defined by the presence of dose-limiting toxicity in 33% of the patients treated at that dose level. Dose-limiting toxicity was considered to be any grade 3 or 4 nonhematologic toxicity as well as grade 2 cardiac ischemia demonstrated by electrocardiography. It was anticipated that at least six patients would be treated at the dose level chosen for

TABLE I. ATP Dose (μg/kg/minute) by Cycle or Retreatment Cycle (ReRx)*

Patient	Age	Cancer site	Cycle 1	Cycle 2	Cycle 3	ReRx 1	ReRx 2	ReRx 3
1	75	Lung	50	50	50			
2	63	Lung	50	50	50[a]			
3	73	Lung	50[b]					
4	48	Lung	50[b]	50[b]				
5	58	Lung	75[b]	50[b]	(50)			
6	57	Maxillary sinus	50	50	50			
7	59	Liver	50	50	pd			
8	72	Lung	50	50	50	100[a]	75[a]	50
9	64	Lung	50	50	50	100[b]	75[b]	75[b]
10	60	Esophagus	50	50	50			
11	62	Lung	50	50	50	100[b]		
12	49	Stomach	75	75	pd			
13	59	Colon	75	75	75			
14	72	Prostate	75	75	pd			

*(50) = theophylline abrogated the toxicity of 50 μg/kg/minute of ATP; pd = treatment discontinued because of progressive disease.
[a]Dose reduced or treatment discontinued due to grade 2 electrocardiographic evidence of ischemia.
[b]Dose reduced or treatment discontinued due to grade 3–4 cardiopulmonary toxicity.

subsequent Phase II testing and that the MTD would be 100 μg/kg/minute based on previously published studies of ATP given as short courses to patients with a variety of diseases other than cancer [14–16].

Patients were examined on at least a daily basis during the ATP infusion and weekly in the outpatient department. Infusions were repeated monthly for a total of at least three infusions, as tolerated by the patient. Patients who completed therapy without serious toxicity and later progressed were eligible for retreatment at a higher dose of ATP, provided they remained eligible based on clinical criteria and signed a new consent form.

Fourteen patients received a total of 43 cycles of ATP therapy, as summarized in Table I. Eleven patients received 29 cycles of treatment at the 50 μg/kg/minute dose level, six patients received 11 cycles of treatment at the 75 μg/kg/minute dose level, and three patients who had tolerated 50 μg/kg/minute very well were reconsented and later given 1 cycle each at the 100 μg/kg/minute dose level. A total of eight (57.1%) patients completed 3 cycles of treatment, seven at the 50 μg/kg/minute dose level and one at the 75 μg/kg/minute dose level.

ATP Whole Blood Measurements

Whole blood ATP levels were measured prior to ATP administration, at 1, 2, 3, and usually 4 hours after starting the infusion, and at 24, 48, 72, and 96 hours while ATP was being infused. Posttreatment levels were obtained a week after the cessation of ATP. Venous whole blood was obtained from the opposite extremity as the infusion line, anticoagulated with either heparin or EDTA, prepared as a lysate, and assayed with duplicate readings of triplicate samples within several minutes of venipuncture by a modification of the luciferin-luciferase method originally described by Beutler and Baluda [17]. The assay was quickly and easily performed using technical details, reagents, and a luminometer (Monolight 1500C luminometer with two injectors) obtained from the Analytical Luminescence Laboratory (San Diego, CA). The mean coefficient of variation between duplicate readings in this study was 1.75% (±0.16% SE). The normal range of whole blood ATP in our laboratory (mean ± 2 SD) based on measurements in 17 patients without cancer and the 8 patients with cancer treated with ATP in this study is 0.56–1.49 mM.

Assessment of Toxicity

Toxicity was evaluated using U.S. National Cancer Institute/Southwest Oncology Group Toxicology Criteria (Division of Cancer Treatment, Bethesda, MD). In this system, cardiac ischemia (chest pain) is graded as follows: 0, none; 1, nonspecific T-wave flattening; 2, asymptomatic, but ST and T wave changes suggest ischemia; 3, angina without evidence of infarction; 4, acute myocardial infarction. Dyspnea is graded as follows: 0, no change; 1, not defined; 2, dyspnea on significant exertion; 3, dyspnea at normal level of activity; 4, dyspnea at rest. Lung toxicity based on the partial pressures of arterial oxygen and carbon dioxide (pO_2/pCO_2) is graded as follows: 0, no change or $pO_2 > 85$ mm Hg and $pCO_2 \leq 40$ mm Hg; 1, $pO_2 > 70$ and $pCO_2 \leq 50$, but not grade 0; grade 2, $pO_2 > 60$ and $pCO_2 \leq 60$, but not grade 0–1; grade 3, $pO_2 > 50$ and $pCO_2 \leq 70$, but not grade 0–2; grade 4, $pO_2 \leq 50$ or $pCO_2 > 70$. In general, toxicity was graded with 1 being mild, 2 moderate, 3 severe, and 4 life-threatening.

Serial measurements of weight and KPS were obtained, as reported previously [18]. Midway through the study, a formal questionnaire was given to the patient at every visit to provide a more objective assessment of KPS, as described by Loprinzi et al. [19].

Statistical Considerations

Statistical analyses were performed using Excel 4.0 spreadsheet software (Microsoft Corporation, Redmond, WA). All P values represent two-tailed values.

RESULTS

Toxicity

The spectrum of toxicity is given in Table II. The first two patients treated with ATP developed asymptomatic hyperuricemia and uricosuria that promptly resolved once ATP was discontinued. There were no other electrolyte changes and no apparent change in tumor status, thus ruling out tumor lysis. All subsequent patients received concurrent allopurinol therapy and hyperuricemia did not recur.

With the exception of cardiac and pulmonary toxicity, and several reactions classified as gastrointestinal or nervous system in origin that occurred along with cardiopulmonary reactions, nearly all of the adverse events seen with ATP were minor (grades 1 or 2). This included hypotension, which occurred during one third of the cycles of therapy. In nearly all patients this consisted of transiently decreased systolic and diastolic blood pressures when the ATP infusion was first started, but this rapidly resolved and only one patient experienced grade 3 hypotension. Serial two-dimensional echocardiography was performed in seven patients (before, 2 or 3 days into the infusion, and, in most cases, a week later). There were no significant differences in heart rate, mean blood pressure, calculated total systemic resistance, cardiac output, left ventricular systolic ejection fraction, and diastolic E to A peak velocity ratio comparing the values on and off ATP.

The dose-limiting toxicities of ATP included asymptomatic electrocardiographic evidence of cardiac ischemia in two patients (no. 2 at 50 and no. 8 at 75 and 100 μg/kg/minute doses) plus grade 3 cardiac toxicity and grade 4 pulmonary toxicity, usually occurring as a characteristic cardiopulmonary syndrome. The typical syndrome included an initial feeling of chest "tightness" (without being called frank pain) that we classified as cardiac in origin, and a sensation of "needing to take a deep breath" that we classified as atypical dyspnea. In most cases these occurred together as a combined cardiopulmonary reaction that resolved with a deep breath or deep yawn and did not progress to frank dyspnea. In other cases, the sensation became sufficiently severe to mandate the cessation of therapy. Only rarely was the cardiopulmonary toxicity accompanied by abnormal arterial blood gas measurements or electrocardiographic changes suggestive of ischemia and none of the patients had a myocardial infarction by electrocardiographic or serum enzyme criteria. All of the episodes of dyspnea at rest (grade 4) resolved promptly by discontinuing therapy and patients did not appear to have a life-threatening problem. These episodes were classified as grade 4 events solely because the chosen toxicity criteria defined all "dyspnea at rest" as grade 4 toxicity.

One patient with preexisting aortic valve disease and advanced nonsmall cell lung cancer developed severe cardiopulmonary toxicity at 75 and again at 50 μg/kg/minute upon retreatment. An additional course of ATP was administered at 50 μg/kg/minute in conjunction with oral theophylline, since theophylline is known to abrogate adenosine toxicity [20]. Pretreatment with theophylline was associated with only minimal (grade 1) pulmonary toxicity. Pretreatment with corticosteroids or antihistamines was not attempted in any of the patients.

The mean pretreatment forced vital capacity (FVC) of patients with dose-limiting cardiopulmonary toxicity was 69.5% compared with 87.9% in those with minor toxicity (normal FVC >80%; $P = 0.025$; Fig. 1). There was no significant difference in the ratio of the FEV_1 divided by the FVC (FEV_1/FVC) or left ventricular ejection fraction values in these two groups of patients.

Serial measurements of weight and KPS were difficult to assess in the group as a whole because of the heterogeneity of diseases involved. The patient with hepatocellular carcinoma had massive ascites and weight changes were uninterpretable. The patient with head and neck cancer and the patient with esophageal cancer couldn't eat because of mechanical obstructions, thus confounding any nonspecific metabolic benefit from ATP. Both weight and KPS remained stable in the patients with nonsmall cell lung cancer.

One patient died shortly after completing three cycles of ATP at a dose of 50 μg/kg/minute. He was a 60-year-old man with advanced esophageal cancer with pulmonary metastases who required frequent dilatations for the treatment of severe gastrointestinal obstructive symptoms. Two days after completing his third course of ATP, which was associated with low-grade fever (101.0°F), intermittent cough, low-grade asymptomatic hypotension (systolic blood pressure 84 mm Hg), low-grade hemoptysis, and anemia that was ascribed to his tumor, the patient collapsed while driving. He was seen at another hospital, where cardiorespiratory resuscitation was unsuccessful and an autopsy was not performed.

MTD

Dose-limiting cardiopulmonary toxicity was seen at all dose levels, but it was dose related and universal at the highest dose level administered (Table II). We had anticipated that the MTD would be 100 μg/kg/minute and all of our patients developed cardiopulmonary toxicity at that dose level. All three patients treated with 100 μg/kg/minute were either disinclined or adamantly refused to continue therapy at that level. Even at 75 μg/kg/minute,

TABLE II. Adverse Events During 43 Cycles of ATP in 14 Patients*

Adverse event	Grade 1 (no.)	Grade 2 (no.)	Grade 3 (no.)	Grade 4 (no.)	Any grade (%)	Grade 3 or 4 (%)
Pulmonary						
Dyspnea	12	3	1	5	49	14
Hypoxia	0	2	2	0	9	5
Cough	1	1	0	0	5	
Hemoptysis	1	1	0	0	5	
Wheezing	1	0	0	0	2	
Hyperventilation	1	0	0	0	2	
Cardiovascular						
Chest pain	5	6	5	0	37	12
Hypotension	12	1	1	0	33	2
ECG abnormality	9	3	0	0	28	
Tachycardia	1	1	0	0	5	
Vasodilation	0	2	0	0	5	
Sweating	0	0	1	0	2	2
Gastrointestinal						
Nausea/emesis	3	1	2	0	14	5
Abdominal pain	1	3	0	0	9	
Constipation	2	0	0	0	5	
Dyspepsia	2	0	0	0	5	
Melena	1	0	0	0	2	
Nervous system						
Dizziness	3	3	1	0	16	2
Headache	2	3	1	0	14	2
Anxiety	1	1	1	0	7	2
Nonspecific pain	1	0	1	0	5	2
Euphoria	1	0	0	0	2	
Insomnia	1	0	0	0	2	
Taste perversion	0	1	0	0	2	
Injection site						
Local reaction	18	2	0	0	47	
Pain at injection site	6	1	0	0	16	
Phlebitis	0	3	0	0	7	
Other						
Fever	6	6	0	0	28	
Pain in back or neck	3	1	0	0	12	
Anemia	2	3	0	0	12	
Leukopenia	3	1	0	0	9	
Hyperuricemia	0	2	0	0	5	
Hypocalcemia	1	0	0	0	2	
Hypomagnesemia	1	0	0	0	2	
Bleeding time increase	1	0	0	0	2	
Conjunctival hemorrhage	1	0	0	0	2	
Thrombocythemia	0	1	0	0	2	
Infection	1	0	0	0	2	
Malaise	0	1	0	0	2	
Rhinitis	0	1	0	0	2	
Total	104	54	16	5	100	21

*All adverse events are included, whether or not they could be ascribed to ATP, except for the posttherapy occurrence of death of uncertain cause in one patient, as described in the text.

50% of the patients developed dose-limiting toxicity, thus placing that dose level above the MTD. Despite cardiopulmonary toxicity, most patients were eager to continue therapy at the 50 and 75 μg/kg/minute dosage levels. Three of 11 (27%) patients developed dose-limiting toxicity during at least one course of treatment at that dose level. Thus, the MTD for ATP given as 96-hour infusions for patients with advanced cancer in this study was 50 μg/kg/minute.

Pharmacokinetics

Interpatient ATP levels vary widely based on differences in nutritional status, hemoglobin level, comorbid conditions, and other factors. Because of this, the pharma-

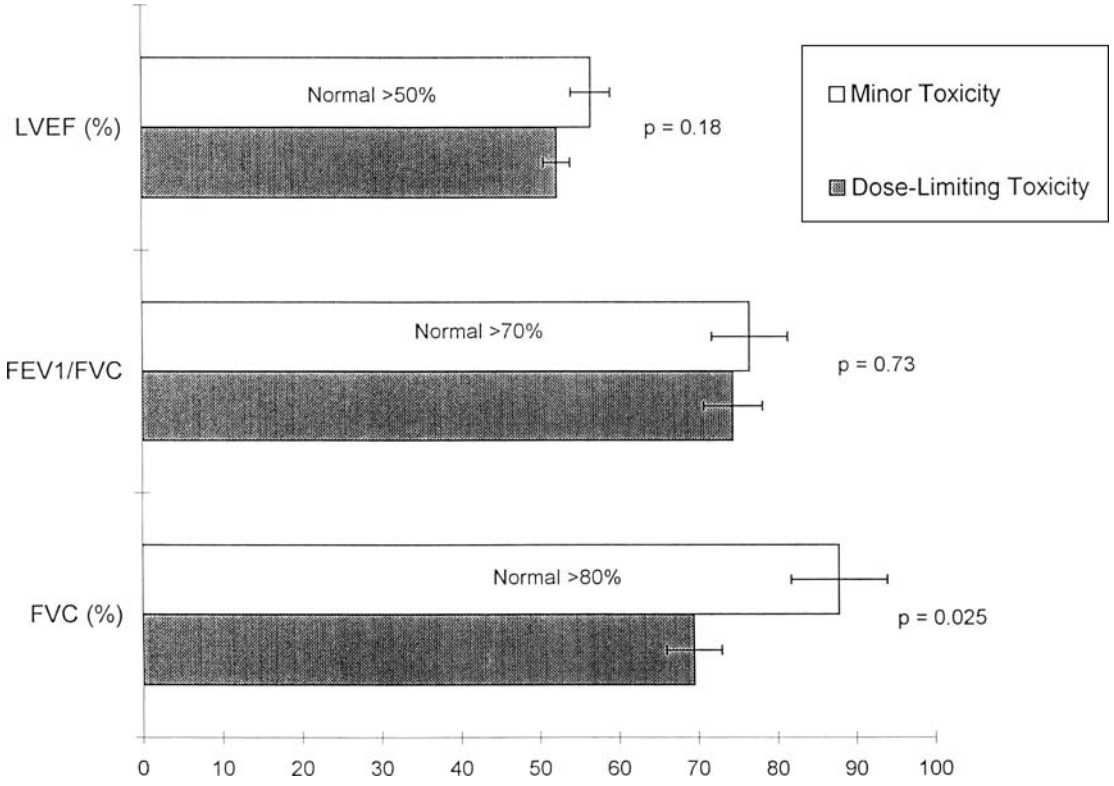

Fig. 1. Left ventricular ejection fraction (LVEF), FEV1/FVC, and FVC in six patients developing dose-limiting cardiopulmonary toxicity compared to eight patients who did not develop this problem while receiving ATP in doses of 50-75 μg/kg/minute.

cokinetic analysis reported here uses each patient as his own control for each cycle of ATP treatment. Eight patients were studied during 18 cycles of therapy at three dose levels. The mean baseline ATP concentration was determined for each patient in triplicate using one or more samples before each course and the result expressed as 100%. The standard error of the baseline levels never exceeded 7%. Serial measurements of ATP on the day of infusion resulted in a maximal increase of 30–40% above baseline by 4 hours, and the levels between 24 and 96 hours maintained a steady state, plateau level above baseline (Fig. 2). The plateau levels above baseline achieved with 50 and 75 μg/kg/minute were 63% and 67%, respectively. Both values were significantly higher than baseline ($P = 0.0006$), but not significantly different from each other. However, these two dose levels were significantly less than the mean plateau level achieved with a dose of 100 μg/kg/minute (116% increase above baseline, $P = 0.0002$ by Z-test assuming unequal variance, comparing the levels achieved with 100 vs. 50–75 μg/kg/minute). One of the patients studied at the 100 μg/kg/minute dose rate also received two courses of ATP at 50 μg/kg/minute. The mean plateau level for this patient was 125% above baseline at 100 μg/kg/minute and 38% and 45% above baseline for the two infusions at 50 μg/kg/minute. These plateau levels were significantly different using a two-sided t-test for paired samples ($P = 0.0044$ and $P = 0.016$, respectively). The second patient studied at the 100 μg/kg/minute dose rate had insufficient separate measurements of ATP blood levels to allow a paired analysis. Whole blood ATP levels returned to or near baseline by 1 week after each infusion.

DISCUSSION

The dose of ATP in this study was solely limited by cardiopulmonary toxicity. In three patients (2, 3, 8) this took the form of cardiac ischemia demonstrated by electrocardiography (grade 2 or 3 cardiac dysfunction). In the other patients, it was a more complex reaction that we classified as grade 3 cardiac and grade 4 pulmonary toxicity, although it is entirely possible that the reaction was in fact solely a pulmonary reaction without a significant cardiac component. The typical syndrome included an initial feeling of chest "tightness" (without being called frank pain) that we classified as cardiac in origin, and a sensation of "needing to take a deep breath" that we classified as atypical dyspnea. In most cases, these occurred together as a combined cardiopulmonary reaction that resolved with a deep breath or deep yawn and did

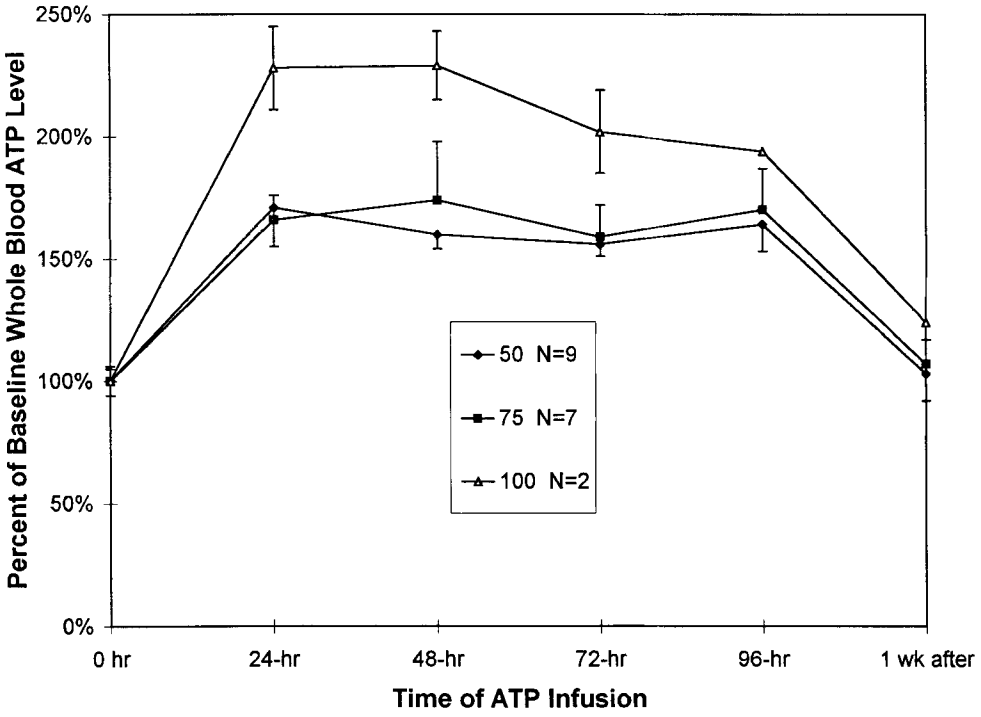

Fig. 2. Mean whole blood levels (± SEM) of ATP using each patient as his own control before, during, and after 96-hour infusions of ATP by dosage level. The plateau level achieved with 100 μg/kg/minute is significantly higher than that achieved with 50–75 μg/kg/minute ($P = 0.0002$).

not progress to frank dyspnea. In other cases, the sensation became sufficiently severe to mandate the cessation of therapy. In every case the severe cardiopulmonary toxicity was marked by easily identifiable symptoms and the patients asked for the discontinuation of treatment. Complete resolution of symptoms followed in a few seconds to a few minutes. This reaction was most pronounced in patients with preexisting reductions in FVC from loss of lung volume and it was also pronounced in two patients with preexisting cardiac disease (aortic valve disease and pericardial effusion). There was no significant difference in airways resistance or left ventricular ejection fraction between those patients with and without cardiopulmonary toxicity.

It is important to draw a sharp distinction between the dose-limiting cardiopulmonary toxicity seen here and the severe cardiopulmonary toxicity reported with other biologic agents, such as interleukin-2 (IL-2). This reflects at least in part a problem in defining grade 4 dyspnea for cancer patients; although technically reaching this level by the National Cancer Institute's (NCI) criterion of dyspnea at rest, the toxicity was rapidly and completely reversed in all patients and did not appear to be dangerous.

We presume that many of the patients with cardiopulmonary toxicity were experiencing adenosine toxicity, since ATP is rapidly converted to adenosine in vivo. Moreover, the plasma half-life of adenosine is very short, namely about 10–15 seconds [20], which is consistent with the time-course of the cardiopulmonary reaction observed. The fact that we were able to prevent cardiopulmonary toxicity in one patient treated with theophylline is consistent with this interpretation.

The sensation of chest tightness and dyspnea has been reported with some other drugs as part of a hypersensitivity reaction. For example, Fossella and colleagues [21] reported such a syndrome in 36% of a group of patients with nonsmall cell lung cancer treated with docetaxel in a Phase II study. Unlike the patients treated with ATP, however, patients with this reaction from docetaxel had a high frequency of flushing, laryngospasm, and wheezing with the reaction. We consider it highly unlikely that hypersensitivity is involved in the cardiopulmonary toxicity of ATP.

Nondose-limiting toxicity was generally minor and insignificant and there was no grade 3 or 4 hematopoietic toxicity. Most patients tolerated ATP well and were eager to continue therapy. One patient died shortly after the administration of ATP. He was a patient with advanced, progressive esophageal cancer who collapsed 2 days after receiving his third and last infusion of ATP at a dose of 50 μg/kg/minute. Unfortunately, the family refused to permit a postmortem examination and the basis of death remains unexplained.

Pharmacokinetic studies of ATP were performed solely

in whole blood rather than plasma or urine for two reasons. First, the extremely short half-life of ATP in plasma makes plasma determinations technically difficult and cumbersome. Second, Rapaport and Fontaine [10] have shown that the administration of ATP to animals results in increased levels of ATP in erythrocytes, and that this results in a marked increase in the steady state level of plasma ATP. Thus, measuring whole blood ATP, which is quick and convenient, is a practical way of determining whether or not ATP infusions result in predictable elevations of blood (and presumably plasma) ATP concentrations. The limitation of this approach, however, is that we were unable to study other pharmacokinetic parameters, such as $T_{1/2}$, volume of distribution, or patterns of metabolic clearance and distribution. Using measurements of whole blood ATP, we confirmed that ATP could be predictably and reproducibly increased in whole blood by infusions, and that a plateau level existed between 24 and 96 hours. These levels were highly significantly greater than baseline, although the plateau levels achieved with 50 and 75 μg/kg/minute were themselves not significantly different. The highest dose level, 100 μg/kg/minute, did achieve levels that were significantly higher than could be achieved with either 50 or 75 μg/kg/minute. This suggests that the whole blood level of ATP may be somewhat controlled at lower levels of ATP administration by physiologic processes, such as by plasma membrane ectonucleotidase, 5′-nucleotidase, and a Na^+-nucleoside cotransporter of ATP [12], but that this can be overcome at higher dose levels.

The MTD for 96-hour infusions of ATP in this study was 50 μg/kg/minute. We had expected the MTD to be 100 μg/kg/minute, based on published results using ATP for short infusion times [14–16]. However, the 100 μg/kg/minute dose rate was excessively toxic when given as a prolonged infusion in this group of patients with advanced cancer. Although higher doses of ATP may prove acceptable in other groups of patients in future trials, such as in women or younger patients of both sexes, our patient population consisted of older men, many of whom had at least some degree of lung volume loss because of lung cancer and its treatment with radiation therapy.

Depending on the nature of the dose-limiting toxicity of a drug, the MTD is not always the most appropriate dose level to choose for subsequent Phase II studies. A lower dose than the MTD is commonly chosen if the dose-limiting toxicity is considered truly life-threatening and severe. In the case of ATP, dose-limiting toxicity rapidly resolves within minutes of stopping therapy, so one might even consider using a higher dose than the MTD for Phase II testing. We do not recommend that, however, since the pharmacokinetic data suggests no substantial increase in whole blood ATP level by increasing the dose to less than 100 μg/kg/minute. Moreover, we anticipate subsequent testing of ATP in combination with other drugs in the future, so it is essential to test a dose level that is likely to be well tolerated during such combination chemotherapy.

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